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Influence of interleukin-18 gene polymorphisms on acute pancreatitis susceptibility in a Chinese population

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ABSTRACT. We investigate the relationship between *IL-18* -607C/A and -137G/C genetic polymorphisms and development of acute pancreatitis in a Chinese population. A total of 153 patients were consecutively recruited from the First Affiliated Hospital of Chongqing Medical University between January 2013 and November 2014. Genotyping of *IL-18* -607C/A and -137G/C variants was performed using the polymerase chain reaction-restriction fragment length polymorphism method. We observed a significant difference between acute pancreatitis patients and control subjects with respect to age (t = 2.15, P = 0.02), gender (chi-square = 3.95, P = 0.04), body mass index (t = 5.85, P < 0.001), and alcohol consumption (chi-square = 9.74, P =

0.002). Using chi-square tests, we found that the genotype distributions of *IL-18* -607C/A (chi-square = 0.81, P = 0.67) and -137G/C (chi-square = 1.16, P = 0.56) polymorphisms did not differ between the acute pancreatitis and control groups. Genotype frequencies of these variants were consistent with Hardy-Weinberg equilibrium in both patient and control groups. In addition, logistic regression analysis failed to identify a significant association between these polymorphisms and acute pancreatitis risk. Our study firstly examined their association in a Chinese population, and we suggest that the *IL-18* -607C/A and -137G/C polymorphisms do not influence susceptibility to acute pancreatitis in the Chinese population studied in the present study.

Key words: *IL-18*; Polymorphism; Acute pancreatitis; Chinese population

INTRODUCTION

Acute inflammation of the pancreas, known as acute pancreatitis, has an estimated mortality rate between 10 and 25%, based on the condition of this disease (Yousaf et al., 2003). The development of acute pancreatitis involves various environmental and lifestyle factors, such as gallstones, heavy alcohol consumption and obstructed pancreatic ducts (Kumaravel et al., 2014; Yuhara et al., 2014; Lankisch et al., 2015). Not all individuals subject to acute pancreatitis risk factors go on to develop this disease, which indicates that genetic influences may contribute to the development of this disease. Recently, many studies have suggested that interleukin (IL) genes, such as *IL-10*, *IL-1β*, *IL-8*, and *IL-6* play an important role in the development of acute pancreatitis (Bao et al., 2015b; Chi et al., 2015; Jia et al., 2015; Li et al., 2015a).

The *IL-18* gene is located on chromosome 11q22.2-q22.3, and including includes six exons and five introns. Genetic polymorphisms capable of altering the function and efficiency of IL-18 may contribute to disease development risk. The single nucleotide polymorphism -607C/A (rs1946518) has been confirmed to have an impact on IL-18 tissue activity and expression (Kalina et al., 2000; Giedraitis et al., 2001). Therefore, we conducted a case-control study to investigate the association between the *IL-18* -607C/A and -137G/C genetic polymorphisms and development of acute pancreatitis in a Chinese population.

MATERIAL AND METHODS

Subjects

A total of 153 acute pancreatitis patients were consecutively recruited from the First Affiliated Hospital of Chongqing Medical University between January 2013 and November 2014. The acute pancreatitis was diagnosed based on a computerized tomography (CT) scan and clinical manifestation. The exclusion criteria were individuals with a history of cancers, serious infectious diseases, malnutrition, endocrine disease, or serious liver or kidney diseases.

A total of 182 healthy controls were recruited, consisting of individuals having received a regular physical examination at our hospital during the same period. All controls confirmed the absence of acute pancreatitis, digestive diseases, or endocrine disease.

Data concerning demographic and lifestyle factors of acute pancreatitis patients and control subjects were collected from medical records or a self-designed questionnaire, and included age, gender, family history of acute pancreatitis, body mass index (BMI), and tobacco and alcohol consumption. The subjects provided their informed consent before participating in our investigation. This study was approved by the Ethics Committee of the First Affiliated Hospital of Chongqing Medical University.

Genotyping analysis

Five peripheral blood samples, collected in vacuum tubes containing 5% ethylenediaminetetraacetic acid, were taken from each study subject. Genomic DNA was extracted using a QIAGEN DNA Blood Mini Kit (QIAGEN, Valencia, CA, USA), following the manufacturer protocol. Genotyping of the *IL-18* -607C/A and -137G/C polymorphisms was performed using the polymerase chain reaction (PCR)-restriction fragment length polymorphism technique. DNA samples were amplified using two different primer pairs specific for these two polymorphic regions of the *IL-18* gene. The primer sequences and PCR products are shown in Table 1. The restriction enzymes for *IL-18* -607C/A and -137G/C were *Mse*I and *Bfu*CI, respectively. The PCR products for *IL-18* -607C/A and -137G/C were 196 and 261 bp, respectively. The cycling conditions were as follows: 94°C for 5 min, followed by 35 cycles of 94°C for 30 s, 61°C for 45 s, and 72°C for 50 s, before a final elongation at 72°C for 5 min.

Table 1. Primer sequences, and polymerase chain reaction products used to genotype *IL-18* -607C/A and -137G/C polymorphisms.

Polymorphism	Primer sequences	PCR product (bp)
-607C/A	5'-CCCTCTCCCCAAGCTTACTT-3' 5'-TTGAGTGGAACAGGAGTCGA-3'	196
-137G/C	5'-TTGTAACATTGTAGGAATTACC-3' 5'-ATGTAATATCAGTATTTTGATGAGA-3'	261

PCR = polymerase chain reaction.

Statistical analysis

Chi-square and Student *t*-tests were taken to analyze the categorical and continuous variables. The Pearson chi-square test was used to analyze statistical differences in *IL-18* -607C/A and -137G/C allele and genotype frequencies between acute pancreatitis patients and control subjects. Departure of *IL-18* -607C/A and -137G/C genotype frequencies from Hardy-Weinberg equilibrium (HWE) was evaluated using chi-square tests. To test the relationship between the *IL-18* polymorphisms of interest and risk of acute pancreatitis, logistic regression analysis was employed to calculate adjusted odds ratios and 95% confidence intervals. SPSS 20.0 (SPSS Inc., Chicago, IL, USA) was used for all data analysis. P values <0.05 were considered statistically significant.

RESULTS

The demographic and lifestyle characteristics of the study subjects are shown in Table 2. Using chi-square or Student *t*-tests, we observed that patients were comparable to controls in terms of family history of acute pancreatitis (chi-square = 1.09, P = 0.29) and tobacco smoking (chi-square = 0.83, P = 0.36). However, we found a significant difference between

these two groups with respect to age (t = 2.15, P = 0.02), gender (chi-square = 3.95, P = 0.04), BMI (t = 5.85, P < 0.001), and alcohol consumption (chi-square = 9.74, P = 0.002).

Table 2. Demographic and lifestyle characteristics of study subjects.

Variable	Patients (N = 153)	%	Controls (N = 182)	%	Chi-square or *t*-test	P value
Age, years	65.86 ± 8.50		63.73 ± 9.42		2.15	0.02
Gender						
Female	55	35.95	85	46.70		
Male	98	64.05	97	53.30	3.95	0.04
Family history of acute pancreatitis						
No	149	97.39	180	98.90		
Yes	4	2.61	2	1.10	1.09	0.29
BMI, kg/m^2	25.36 ± 3.58		23.17 ± 3.27		5.85	<0.001
Tobacco smoking						
No	97	63.40	124	81.05		
Yes	56	36.60	58	37.91	0.83	0.36
Alcohol consumption						
No	86	56.21	132	86.27		
Yes	67	43.79	50	32.68	9.74	0.002

BMI = body mass index.

The distributions of *IL-18* -607C/A and -137G/C genotypes are presented in Table 3. Among patients, 96 (62.75%), 45 (29.41%), and 12 (7.84%) individuals carried the CC, CA, and AA genotypes of *IL-18* -607C/A, respectively, while 40 (26.14%), 74 (48.37%), and 39 (25.49%) were found to have the GG, GC, and CC genotypes of *IL-18* -137G/C, respectively. In the control group, 122 (67.03%), 49 (26.92%), and 11 (6.04%) subjects were observed to have the CC, CA, and AA genotypes of *IL-18* -607C/A, respectively, while 57 (31.32%), 82 (45.05%), and 42 (23.63%) carried the GG, GC, and CC genotypes of *IL-18* -137G/C, respectively. Chi-square tests failed to reveal significant differences in the genotype distributions of the -607C/A (chi-square = 0.81, P = 0.67) and -137G/C (chi-square = 1.16, P = 0.56) polymorphisms between the study groups. The genotype frequencies of these variants did not depart from HWE in either the patient or the control group. Using logistic regression analysis, we found no significant association between the *IL-18* -607C/A and -137G/C polymorphisms and acute pancreatitis risk.

Table 3. Relationship between *IL-18* -607C/A and -137G/C polymorphisms and acute pancreatitis risk.

IL-18	Patients (N = 153)	%	Controls (N = 182)	%	Chi-square	P	P for HWE		OR (95%CI)[1]	P
							Patients	Controls		
-607C/A										
CC	96	62.75	122	67.03					1.0 (Ref.)	
CA	45	29.41	49	26.92					1.17 (0.70-1.95)	0.53
AA	12	7.84	11	6.04	0.81	0.67	0.06	0.06	1.39 (0.53-3.63)	0.46
Allele										
C	237	77.45	293	80.49					1.0 (Ref.)	
A	69	22.55	71	19.51	0.93	0.33			1.20 (0.81-1.78)	0.33
-137G/C										
GG	40	26.14	57	31.32					1.0 (Ref.)	
GC	74	48.37	82	45.05					1.29 (0.75-2.22)	0.34
CC	39	25.49	43	23.63	1.16	0.56	0.69	0.24	1.32 (0.70-2.50)	0.36
Allele										
G	154	50.33	196	53.85					1.0 (Ref.)	
C	152	49.67	168	46.15	0.97	0.33			1.17 (0.85-1.60)	0.33

[1]Adjusted for age, gender, body mass index, and alcohol consumption. HWE = Hardy-Weinberg equilibrium, OR = odds ratio, CI = confidence interval, Ref. = reference.

DISCUSSION

In the present study, we evaluated the relationship between the *IL-18* -607C/A and -137G/C polymorphisms and acute pancreatitis risk in a Chinese population, finding that these sequence variations did not contribute to the development of this disease.

Previous studies have reported that *IL-18* sequence variations are associated with several diseases, including tuberculosis, rheumatoid arthritis, Crohn's disease, *Helicobacter pylori* infection, ischemic stroke, hepatitis B virus (HBV)-related liver disease, hepatocellular carcinoma, and other cancers (Liu et al., 2013; Angelo et al., 2015; Bao et al., 2015a; Gao et al., 2015; Karra et al., 2015; Li et al., 2015b; Myung et al., 2015; Shi et al., 2015; Zhou et al., 2015). Zhou et al. (2015) examined the association between the polymorphisms under investigation in the present study and tuberculosis, reporting that the -137G/C variant contributes to susceptibility to this disease in the Han Chinese population. In a study of a Brazilian population, Angelo et al. (2015) suggested a possible role for *IL-18* polymorphism in rheumatoid arthritis, while Gao et al. (2015) indicated that variants of this gene may contribute to susceptibility to Crohn's disease in Asians and Africans. Bao et al. (2015a) conducted an investigation involving 153 patients and 165 healthy controls from a Chinese population, finding that the *IL-18* -137G/C polymorphism may play a protective role in hepatocellular carcinoma. In addition, Myung et al. (2015) suggested that *IL-18* may contribute to the pathogenesis of *H. pylori*-associated diseases. A meta-analysis of 29 studies performed by Li et al. (2015b) revealed the *IL-18* -607C/A polymorphism to correlate with a significantly increased risk of breast cancer, nasopharyngeal carcinoma, and esophageal cancer. Moreover, Shi et al. (2015) conducted a study of 322 Chinese ischemic stroke patients and 322 controls, demonstrating that *IL-18* -607C/A influences the development of this condition, while Karra et al. (2015) reported that this same polymorphism may confer protection against HBV infection in an Indian population. These studies indicate that *IL-18* genetic polymorphisms may contribute to the development of various diseases.

Currently, only one study have examined the role of serum concentrations of *IL-18* in the prognosis of acute pancreatitis (Janiak et al., 2015). Janiak et al. (2015) carried out a study of 32 patients with acute pancreatitis, suggesting that IL-18 level increases in the initial phase of acute pancreatitis. However, no study have investigated the association between *IL-18* polymorphism and development of acute pancreatitis. The exact molecular mechanisms underlying the pathogenesis of acute pancreatitis remain to be investigated in further studies.

Two limitations of the present study should be taken into account. First, selection bias may have been present, as all patients and control subjects were recruited from one hospital. Second, other genes that may influence the development of acute pancreatitis were not considered in our analysis.

In summary, no previous studies have examined the relationship between *IL-18* polymorphisms and acute pancreatitis risk until now. Our study firstly examined their association in a Chinese population, and we suggest that the *IL-18* -607C/A and -137G/C polymorphisms do not influence susceptibility to acute pancreatitis in the Chinese population studied in the present work. Further investigations with large sample size on the molecular mechanisms underlying the pathogenesis of acute pancreatitis are greatly requires.

Conflicts of interest

The authors declare no conflict of interest.

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